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BRIEF COMMUNICATION

Endotoxin Treatment of Pregnant Rats Affects Sexual Behavior of the Male Offspring

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WIJKSTRA, S., N. VALKHOF, J. M. KOOLHAAS AND G. A. SCHUILING. *Endotoxin treatment of pregnant rats affects sexual behavior of the male offspring.* PHYSIOL BEHAV **49**(3) 647–649, 1991.—The offspring of endotoxin-infused pregnant rats (0.2 μg endotoxin, 53.3 min, day 18 of pregnancy) did not exhibit different behavior in the Hebb-Williams-type maze test, but the males showed aberrations in the sexual behavior test. Because endotoxin did not cross the placental barrier, it was concluded that the effect reflects abnormal brain development, caused by endotoxin-induced placental malfunction, notably impaired oxygen transport.

Rat Pregnancy Endotoxin Placental damage Offspring Maze test Sexual behavior

IN many species, including man and the rat, placental malfunction, notably diminished oxygen transport, is generally held to be a main cause of malformation and even abortion of the fetuses (2). Moreover, lack of oxygen may result in damage of the central nervous system with consequent aberrations of the behavior of the offspring later in life (3,6).

Recently we demonstrated by means of pulse-oximetry in fetal rats that the oxygen transport by the placenta was seriously diminished after treatment of the mother with endotoxin as low as 0.2 μg on day 19 of pregnancy (13). While higher doses of endotoxin induce abortion and resorption of the fetuses [(7,9), own observations], the above dose generally does not interfere with a normal delivery of the offspring on day 21 of pregnancy. Yet, also after 0.2 μg of endotoxin, characteristic pathology in the placenta (1) could be demonstrated.

In mice (5) it was demonstrated that after treatment of the mother with high doses (2.5 or 5 μg) of endotoxin, the offspring exhibited neuronal necrosis in the diencephalon and spinal cord and showed aberrations in the audiogenic seizure response at the age of 28 days.

In this study pregnant rats were infused on day 18 with 0.2 μg endotoxin and the offspring were tested using maze and sexual behavior tests at the age of 12–13 weeks. In another experiment 0.2 and 2.0 μg endotoxin were infused and endotoxin concentrations were measured in the maternal and fetal blood in order to assess possible placental transport of endotoxin.

METHOD

Four-day cyclic rats of the local Wistar strain were kept in a temperature- and light-controlled room with free access to food and water (light: 0700–1900 h). Daily vaginal smears were taken. Proestrous rats were rendered pregnant by housing them at the age of 4 months with fertile males for one night (next day = day 0 of pregnancy). Rats were cannulated on day 0 of pregnancy according to Steffens (11). Endotoxin in pyrogen-free saline (*E. coli*, Whittaker M.A. Bioproducts Inc., Walkersville, MD) or saline alone was infused into the conscious animal under stress-free conditions on day 18 of pregnancy. Endotoxin (0.2 or 2.0 μg) was infused at the rate of 0.0375 ml/min for 53.3 min (E-rats). Control rats received the same volume of saline (S-rats). Some rats were sacrificed immediately after infusion for determination of endotoxin in maternal and fetal blood (blood of 7 to 10 fetuses was pooled) in the Limulus Amebocyt Lysate test (12). The remaining rats delivered on day 21. Pups were nursed by their own mothers. At weaning on day 21 of lactation the lighting schedule was changed to light: 2030–0830 h. At the age of 6 weeks males and females were housed separately.

Behavior tests took place at the beginning of the dark period under dimmed light conditions. At the age of 12 weeks pups were tested in a Hebb-Williams-type maze and during 10 min the frequency of rearing and crossing was registered (♀ S-rats n = 12, ♂ S-rats n = 17, ♀ E-rats n = 15, ♂ E-rats n = 14). One week

later male pups were tested in a neutral cage (80 × 55 × 50 cm). After 5 min an estrous female was presented. During the next 10 min the males' (15 pups of S-rats and 14 pups of E-rats) behavior was registered. Frequency and percentages of time (10 min = 100%) of the following categories of sexual behavior were registered: investigate, mount, follow, intromission, ejaculation, penis groom, groom and explore. For the categories mount, intromission and ejaculation, latencies were recorded. If an animal did not exhibit mount, intromission or ejaculation within the 10 min of the test, the score was considered to be 100% = 600 s.

Statistical comparison between the E and S groups were made by the Mann-Whitney U-test. For the ejaculation response comparison between both groups was made by a 2 × 2 test of independence. Statistical comparison of more than two groups was made by analysis of variance (Kruskal-Wallis); subsequently groups were compared in twos by the Mann-Whitney U-test (10). Differences were considered to be statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

In the Hebb-Williams-type maze test no differences were found in the frequency of crossing and rearing between E- and S-rats. Sexual behavior, on the other hand, was changed in E-rats (see Fig. 1). With the exception of the relative time spent on groom (an essentially nonsexual behavior; E-rats spent more time on groom $p<0.05$; Mann-Whitney U-test), no differences were found in frequency and relative time spent on the various behavior elements between S-rats and E-rats. All S-rats (n = 15) exhibited mount, intromission and ejaculation, but only 12 out of 14 E-rats exhibited intromission of which 5 ejaculated ($p<0.01$; 2 × 2 test of independence). The latencies for mount, intromission and ejaculation, however, were increased in E-rats ($p<0.05$; Mann-Whitney U-test).

After 53.3 min of endotoxin infusion of the mothers with 0 μg (saline, n = 4), 0.2 μg (n = 5) or 2.0 μg (n = 4), no endotoxin was measured in fetal blood (detectable level 50 pg/ml), while in the maternal blood the endotoxin concentration rose with increasing endotoxin infusion rates: saline<50 pg/ml; 0.2 μg endotoxin: 1670 ± 200 pg/ml; 2.0 μg endotoxin: 6800 ± 1490 pg/ml (Kruskall-Wallis test statistic = 10.74; Mann-Whitney U-tests: $p<0.05$ for all 3 comparisons). From these data it was concluded that endotoxin does not cross the placental barrier and that the behavioral effects of endotoxin treatment were not due to direct effects of endotoxin on the fetal brain.

It is more likely that behavioral aberrations observed in this study result from some damage inflicted to the brain of the offspring [cf. (5,9)] due to placental malfunction (1), notably impaired oxygen transport to the fetuses (13). Indeed, lack of oxygen may result in aberrations in behavior of the offspring, e.g., maze learning (3). Yet, because in this study pups were left with their (endotoxin-treated) mothers, it cannot be entirely excluded that the behavior aberrations result from disturbed maternal behavior, since it has been shown that decreased postnatal behavior of the mothers (licking of the pups) may result in affected sexual behavior of the male offspring (8).

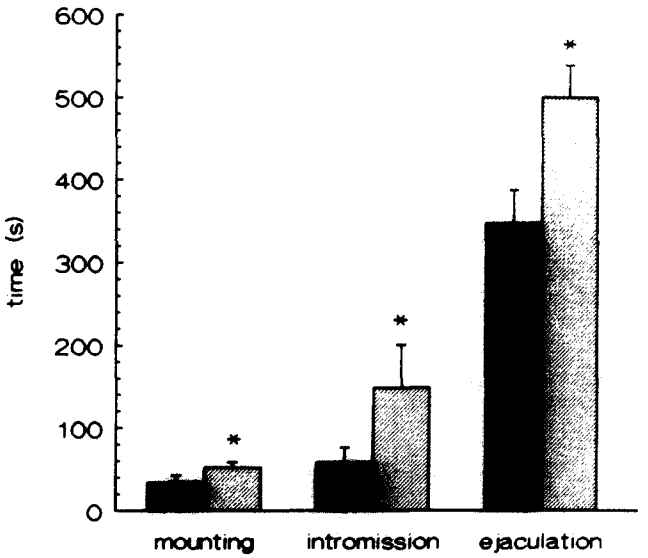

FIG. 1. Latencies (mean ± SEM) of mounting, intromission and ejaculation displayed during the sexual behavior test. Solid bars: offspring of saline infused rats (n = 15); shaded bars: offspring of endotoxin infused rats (n = 14). *Significantly different from S-rats ($p<0.05$, Mann-Whitney U-test).

The endotoxin-treated pregnant rat may be a useful model for the study of the development of the neuronal substrate underlying behavioral complexes which play a role in later life. The fact that (certain elements of) sexual behavior but not exploring behavior is affected after treatment of rats with endotoxin on day 18 of pregnancy may suggest that the neuronal substrate underlying sexual behavior, but not that underlying exploring behavior, is in a critical stage of development at that time. In other words, there may exist "critical periods" for the development of various complexes of behavior. Indeed, developmental neurologists have demonstrated that in the human there is a fixed sequence in the appearance of behavioral patterns (4). Lack of oxygen at the onset of the development of the pertinent neural substrate may be a major cause of perturbations in its function in later life.

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